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Microradiographic study on the effects of mucin-based solutions used as saliva substitutes on demineralised bovine enamel in vitro

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Summary Sialic acids and proteins bound to mucins are known to form complexes with calcium, and this mechanism may hamper the remineralisation of calcium-containing mucin-based saliva substitutes. Thus, the aim of this investigation was to evaluate the effects of adding various concentrations of calcium phosphate to self-made mucin-containing solutions on demineralised bovine enamel in vitro.

Bovine specimens were prepared, embedded in epoxy resin, and polished to 4000 grit. Subsequently, the surfaces of the specimens were partially covered with nail varnish, thus serving as a control of sound enamel, and demineralised (37 °C; pH 5.0) for 14 (19 groups; $n = 10$) or 28 days (three groups; $n = 9$). After demineralisation, the specimens were exposed to mucin-based solutions (30 g/l) with various saturations with respect to apatites containing 0.1 mM NaF, $CaCl_2$ (0—20 mM) and KH_2PO_4 (0—52 mM) at two different pH values (5.5 or 6.5). A fluoride-free solution and the commercially available saliva substitute Saliva Orthana® (Orthana, Kastrup, Copenhagen Denmark) served as controls. The differences in mineral loss ($\Delta\Delta Z$) between the values prior to (ΔZ_{Demin}) and after storage (ΔZ_{Effect}) in the various solutions were evaluated from microradiographs of thin sections (100 μm).

The general linear model revealed a significant dependency of $\Delta\Delta Z$ for calcium ($P = 0.006$), but not for phosphate ($P = 0.081$) or pH ($P = 0.114$). ΔZ_{Effect} was only

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significantly reduced compared with ΔZ_{Demin} in the group with the highest saturation with respect to hydroxyapatite ($P < 0.05$; t-test).

In conclusion, mucin-based saliva substitutes with an adequate composition are able to remineralise bovine enamel in vitro.

Introduction

Patients suffering from hyposalivation after therapeutic irradiation to the head and neck show difficulties with swallowing, speech, taste and mastication. In these cases, quantitative and qualitative changes of the protective salivary films or pellicles that coat dental hard tissues can be observed.[1] Mixed saliva in humans mainly consists of proteins, lipids and water. Several proteins, such as statherin, histatins, proline-rich proteins and mucins, are known to influence the mineralisation of enamel and dentine. For instance, mucins, high-molecular-weight glycoproteins that account for 7—26% of total salivary proteins,[2,3] seem to contribute to the inhibition of demineralisation against erosive attacks on enamel.[4] Moreover, in the presence of fluoride, calcium diffusion through a mucin film seems to be enhanced.[5] This mechanism was also found to support enamel remineralisation by physiological concentrations of mucin in vitro.[6]

In patients with diminished salivary flow, increased incidence of caries can be observed unless strong preventive efforts are applied.[7] Saliva substitutes mainly based on carboxymethylcellulose (CMC), mucin or linseed are administered to improve the oral complaints and to support caries prevention.[8] Nevertheless, some of the marketed products seem to have a demineralising effect on dental hard tissues.[9,10] The addition of polymers such as mucins or CMC increases the viscosity of these solutions compared with human saliva, which may hamper the remineralising properties of these solutions. On the other hand, increased calcium and phosphate concentrations may enhance rehardening properties.[11,12]

Nevertheless, the accessible literature only provides scanty information regarding how the remineralising potential of mucin-based artificial saliva can be increased by the addition of various ratios of calcium phosphates. For aqueous solutions, the degree of saturation with respect to apatites can be obtained by software calculation if the pH and the concentrations of certain ions are known.[13] For solutions containing organic components or higher viscosities, this calculation may not provide a predictable outcome of their de- and remineralising effects on dental hard tissues. Thus, the present investigation tested the null hypothesis that mineral losses of enamel specimens are comparable for the variables 'calcium', 'phosphate' and 'pH' after storage in various mucin-containing solutions that differed in these variables.

Materials and methods

Sample preparation

The crowns of freshly extracted bovine incisors were separated clearly below the cemento-enamel junction using a diamond-coated band saw under continuous water cooling (Exakt 300cl; Exakt Apparatebau, Norderstedt, Germany). From each crown, four slabs (approximately 3 mm × 4 mm × 2 mm) were prepared from the labial aspect and embedded into epoxy resin (Technovit 4071; Kulzer, Wehrheim, Germany). The specimens were ground flat and hand-polished to 4000 grit (silicon carbide; Diaplus, Oststeinbeck, Germany), thereby removing about 200—400 μm of the outer enamel layer. One-third of each specimen's surface was partially covered with nail varnish to serve as a control. Subsequently, the specimens were demineralised in a solution containing 6 μM methylhydroxydiphosphonate, 3 mM $CaCl_2 \times 2H_2O$, 3 mM KH_2PO_4, 50 mM acetic acid and vestiges of thymol for 14 days (n = 190) or 28 days (n = 27) at pH 5.0 in an incubator (Wärmeschrank BR 6000; Heraeus Kulzer, Germany; 37 °C). The pH value was checked daily (pH-Meter CG 819; Schott Geräte, Hofheim, Germany) and slight elevations were corrected with lactic acid to maintain a constant pH value between 5.0 and 5.1 during the demineralisation period.

In vitro exposure

After 14 days of demineralisation, 190 specimens were divided into 19 groups (n = 10) and exposed to self-made solutions differing in $CaCl_2$ (0—2 mM) and KH_2PO_4 (0—13 mM) concentrations at two pH values (5.5 or 6.5). The solutions also contained 0.1 mM sodium fluoride. A fluoride-free solution and the commercially available saliva substitute Saliva Orthana® (Orthana, Kastrup, Copenhagen, Denmark) served as controls (part 1). The 27 specimens that were demineralised for 28 days (part 2; n = 9) were exposed to one of two fluoride-containing